

METHOD AND SYSTEM FOR DETECTING SENTINEL LYMPH NODES

RELATED APPLICATIONS

This application claims the benefit of serial number 60/395,279, entitled "Detecting Sentinel Lymph Nodes," filed provisionally on July 12, 2002.

5 This application also claims the benefit of serial number 60/_____, entitled "Method and System for Near-Infrared Fluorescence Contrast-Enhanced Imaging with Area Illumination and Area Detection," filed provisionally on June 20, 2003.

10 GOVERNMENT RIGHTS

This invention was made with Government support from the National Institute of Health, Contract Nos. R01 CA67176 and R21 CA 96305-01. The government may have certain rights in this invention.

15 TECHNICAL FIELD OF THE INVENTION

This invention relates generally to the field of medical imaging and, more specifically, to a method and system for detecting sentinel lymph nodes for surgical resection and prognostic indication of cancer spread through lymph flow.

BACKGROUND OF THE INVENTION

The status of the lymph nodes serves as the most important prognostic indicators for patients with breast carcinoma and provides one of the parameters that indicates the need for adjuvant treatment. Breast cancer patients with mammographically detected cancers first undergo diagnostic lymphoscintigraphy. Lymphoscintigraphy is typically performed using a radiocolloid as a nonspecific contrast agent injected peritumorally for nuclear imaging and localization of sentinel or closest lymph nodes for surgical resection and subsequent biopsy. The resection and biopsy of the sentinel rather than major lymph nodes minimize surgical evasiveness.

However, in breast cancer patients, lymphoscintigraphy is compromised by increased amounts of adipose tissue in obese subjects, where the sentinel lymph location can be as deep as 4 cm from the tissue surface. Nuclear imaging is also hampered by chemotherapy, which may shrink the size of the lymph node to as small as 5 mm. In addition, nuclear imaging may not provide adequate sentinel node detection because of slow and inconsistent drainage of radiopharmaceuticals through the lymphatic system.

U.S. Patent No. 5,865,754 to Sevic-Muraca et al. describes a fluorescence imaging system that relates to in vivo imaging of biologic tissue by mapping a fluorescence characteristic of the tissue through the detection of light emitted in response to excitation light from a time-varying light source.

SUMMARY OF THE INVENTION

According to one embodiment of the present invention, a method for detecting lymph nodes in a human includes introducing a fluorescent contrast agent into a lymph node system of a body, directing near-infrared time-varying excitation light into the tissue of the body, causing the near-infrared time-varying excitation light to contact a lymph node of the lymphatic system, whereby a redshifted and time-varying emission light is generated, detecting the time-varying emission light at a surface of the body, filtering the time-varying emission light to reject excitation light re-emitted from the lymph node, and imaging the lymph node of the lymphatic system.

Embodiments of the invention provide a number of technical advantages. Embodiments of the invention may include all, some, or none of these advantages. In one embodiment, fluorescence-enhanced near-infrared optical imaging measurements provide localization of small targets at substantial tissue depths. This leads to the utilization of nonradioactive tracers for the management of cancer and other diseases, which eliminates the need for potentially harmful nuclear imaging methods that employ ionizing radiation. Fluorescent contrast agents may be used to provide signals for localization of sentinel lymph nodes and, depending on the type of contrast agent, may provide an assessment of lymph node status (i.e., whether or not cancer cells are present), thereby eliminating the need for surgical resection.

Other technical advantages are readily apparent to one skilled in the art from the following figures, descriptions, and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present invention and its features and advantages, reference is now made to the following description, taken in conjunction with the accompanying drawings, in which:

5 FIGURE 1 is a schematic depicting lymphatic structure near a breast of a human;

FIGURE 2A is a schematic illustrating a frequency-domain photon migration system and resulting propagation of a spherical excitation wave and the generated emission wave, as well as the scattered excitation wave;

10 FIGURES 2B through 2E are schematics similar to FIGURE 2A illustrating the propagation of other types of excitation waves;

FIGURE 3 is a flowchart illustrating a method for detecting lymph nodes according to one embodiment of the present invention;

15 FIGURES 4A and 4B are schematics illustrating the nominal intensity values of an incident modulated excitation wave, the generated emission wave that propagates to the surface and the amplitude in the measured wave if ambient, nonmodulated light were also collected;

20 FIGURE 5 is a Jablonski diagram illustrating radiative and non-radiative relaxation of fluorophores to the ground state according to an embodiment of the invention;

FIGURE 6 is a schematic illustrating a system for excitation area illumination and area detection using frequency-domain photon migration ("FDPM") measurements according to an embodiment of the present invention;

25 FIGURES 7A through 7D are DC and AC (a.u.) contrast images (background subtracted) that were simultaneously obtained when a 100 μ L target containing 100 fmol of ICG was positioned at increasing depths within 1% Liposyn[®] in increments of 1 cm from the illumination surface; and

FIGURES 8A and 8B are DC (a.u.) and AC (a.u.) images, respectively, of the phantom scattering surface with no fluorescent target present.

DETAILED DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a schematic of a lymphatic structure near a breast 100 of a human. The use of a less invasive sentinel lymph node biopsy to stage the status of the axillary lymph nodes 102 is desirable for health and safety reasons. Sentinel lymph node biopsy involves removing the sentinel lymph node, which is the first node to which a primary tumor drains before draining into the major node, such as the axillary or internal mammary lymph nodes (as denoted by reference numeral 104). Since lymphatic dissemination of breast carcinoma occurs in a stepwise fashion, the sentinel node is thought to be the first lymph node in which metastasis can be found. If no metastases are found in the sentinel lymph node, it is likely that no metastases are present in the remaining axillary and internal mammary lymph nodes to which it drains. Negative predictive values of greater than 95% have been obtained with sentinel lymph node biopsies. Furthermore, researchers have demonstrated that the status of sentinel lymph node accurately predicts the metastatic status of the remaining axillary nodes with positive predictive values of 100%. Thus, a sentinel lymph node biopsy may obviate unnecessary extensive dissection of the axillary lymph node basin in over half of all patients.

Currently, there are two main methods used to detect the sentinel lymph node: (1) lymphoscintigraphy and (ii) sentinel lymph node mapping. In lymphoscintigraphy, a radiocolloid, generally technetium-99m, is injected peritumorally and the breast is massaged. The tracer slowly is taken up by the lymph and transits to the sentinel node and then to the axillary or internal mammary nodes. During this time, the gamma emission given off by the technetium colloid is collected to provide a gamma image providing a gross location of the draining lymph nodes. The second approach is termed sentinel lymph node mapping. In this procedure performed interoperatively, the sentinel lymph node is detected intraoperatively either visually after the injection of blue dye (isosulfan blue) at the site of the primary tumor or by nuclear imaging or scanning with a handheld gamma probe following injection of a radiopharmaceutical, such as technetium-99m sulfur colloid. Both methods of administering the radiopharmaceutical and the isosulfan blue dye can be used independently or concurrently with success rates varying between 80% - 95%. The success rate at any particular institution, however, is highly dependent upon the

surgeons' experience. In addition, the localization of the sentinel node is more difficult and less successful in patients with fatty tissue as well as those undergoing adjuvant therapy. In the latter case, the lymph nodes can be significantly shrunk, and more difficult to localize.

5 According to the teachings of one embodiment of the present invention, near-infrared ("NIR") frequency-domain photon migration ("FPDM") imaging techniques coupled with fluorescent contrast agents facilitates imaging and localization of the sentinel lymph nodes. Other suitable imaging techniques may be utilized for imaging of lymph nodes, such as magnetic resonance imaging using, among other suitable
10 agents, iron oxide contrast agents; or ultrasound using microbubble emulsions. However, unlike these conventional techniques that require tissue concentrations of exogenous contrast agents at millimolar concentrations, both nuclear and fluorescence optical imaging techniques require orders of magnitude lower tissue concentrations to generate images. This enables the opportunity for molecularly targeted fluorescent
15 contrast agents that specifically bind to cancer cells within the lymphatic system. Specificity is achieved by chemically conjugating the fluorescent molecule to a ligand that specifically binds to an overexpressed receptor or is acted upon by enzymes specific to the cancer cells. The use of fluorescence agents for imaging for sentinel lymph node is accomplished using imaging techniques such as those techniques described in
20 U.S. Pat. No. 5,865,754, which is herein incorporated by reference for all purposes. In another embodiment, when these molecularly targeting fluorescent agents are conjugated with suitable targeting peptides or polymers with enzyme-specific cleavage sites that are specific to molecular markers of cancer, fluorescent signals provide an assessment of lymph node status in situ, thereby eliminating the need for
25 dangerous surgical resection.

 It should be noted that the use of fluorescent agents is not limited to imaging the lymphatic drainage of the breast, but may be used for cases of melanoma, colon cancer, and an increasing number of solid tumor cancers, among others.

 FIGURE 2A is a schematic of an FPDM system 200 illustrating the
30 propagation of a spherical excitation wave 202 and a generated emission wave 204, as well as a scattered excitation wave 206. Briefly, FPDM involves launching spherical excitation wave 202 of near-infrared ("NIR") light from one or more laser diodes 208

into the tissue of a body of interest. In other embodiments, excitation wave 202 may be an array of spherical waves that illuminate the tissue boundary, as denoted by reference numeral 250 in FIGURE 2B, planar (area) waves from an expanded beam of light, as denoted by reference numeral 252 in FIGURE 2C, spherical distributions of concentric circles, as denoted by reference numeral 254 in FIGURE 2D, or a ronchi rule pattern, as denoted by reference numeral 256 in FIGURE 2E. Excitation wave 202 may be intensity-modulated as produced by a frequency generator 210 driving laser diode 208 or other suitable NIR light source. Since NIR light of approximately 700-900 nm wavelength can penetrate several centimeters of tissue without the harmful radiation effects of gamma rays, x-rays, or ultraviolet light, it is advantageous for biomedical imaging and may be used to image as deep as five centimeters or more beneath a surface 212 of the tissue.

Various techniques for NIR fluorescence contrast-enhanced imaging with area illumination and area detection are also illustrated in U.S. Provisional Patent Application No. 60/_____, filed June 20, 2003, which is herein incorporated by reference for all purposes.

As shown in FIGURE 2A, NIR excitation wave 202 propagates through the tissue and towards a target body 214, such as a lymph node. When excitation wave 202 contacts target body 214, which has been enhanced by a suitable fluorescent contrast agent, as described in greater detail below, excitation wave 202 causes generated red-shifted emission wave 204, as well as scattered fluorescent wave 206. Emission wave 204 then propagates towards surface 212 and is detected by a detector 216 as it exits surface 212. Detector 216 may be any suitable detector, such as an intensified charge coupled device ("CCD") camera, photon multiplier tube, or photodiode capable of detecting the intensity modulated fluorescent light. Target body 214 is then imaged in any suitable manner. Examples of such imaging techniques are aptly described in U.S. Pat. No. 5,865,754. An example FPDM imaging technique is described with reference to the flowchart of FIGURE 3.

FIGURE 3 is a flowchart illustrating an example method for detecting lymph nodes according to one embodiment of the present invention. The example method begins at step 300 where a fluorescent contrast agent is administered to the patient and introduced into a lymphatic system of a body of a human. Any suitable fluorescent

contrast agent may be utilized; however, a preferred fluorescent contrast agent is indocyanine green ("ICG") and its derivatives. ICG is a compound with FDA approval for systemic administration in humans. In one embodiment, ICG is excited at 780 nm and emits at 830 nm, has an extinction coefficient of $130,000 \text{ M}^{-1} \text{ cm}^{-1}$, a
5 fluorescent lifetime of 0.56 ns and a quantum efficiency of 0.016 for the 780/830 nm excitation/emission wavelengths. At step 302, intensity modulated NIR excitation light is directed into the tissue of the body. In one embodiment, the NIR excitation light has a wavelength between approximately 700 and 900 nm. Any suitable modulation frequency may be utilized for the intensity modulated NIR excitation
10 light; however, in one embodiment, the modulation frequency is approximately 100 MHz.

The excitation light is caused to contact a node of the lymphatic system, as denoted by step 304. In one embodiment, a sentinel lymph node is contacted. This generates a red shifted and time-varying emission light from the lymph node, as
15 denoted by step 306. This generated emission light propagates through the tissue to the surface of the body where it is received, as denoted by step 308. The emission light is then collected and passed through one or more optical filters that are selected to reject the excitation portion of the re-emitted light signal, as denoted by step 310. The detected fluorescence wave is both phase shifted by θ and AC amplitude
20 attenuated relative to the incident excitation light because of both the propagation of excitation and emission light within the tissue as well as the relaxation kinetics of the fluorophore. The lymph node may then be imaged based on the filtered emission light, as denoted by step 312, which then ends the example method in FIGURE 3.

FIGURES 4A and 4B are schematics illustrating the nominal intensity values
25 of an incident modulated excitation wave, the generated emission and excitation waves that propagate to the surface, and the amplitude in the measured excitation wave if ambient, non-modulated light were also collected according to an embodiment of the present invention. Unlike continuous wave techniques, the contribution of background light to the detected fluorescent signal is eliminated from
30 the AC and θ components because of the inherent filtering of non-modulated light using FDPM. This rejection of non-modulated light through FDPM techniques results in a reduction of the measurement noise floor, enabling greater penetration depth into

the tissue and more sensitive detection of fluorescent targets. However, as with continuous wave techniques, inadvertent collection of propagated excitation light increases the measurement noise floor and reduces sensitivity and depth of penetration.

5 With reference to FIGURES 4A and 4B, a snapshot of an intensity modulated (100 MHz) excitation light 400 and an attenuated excitation wave 402 detected at a distance of approximately two centimeters from the source is illustrated. The scattering properties of the solution are $\mu_s' = 10 \text{ cm}^{-1}$ and $\mu_a = 0.1 \text{ cm}^{-1}$. If there is a change in the ambient light levels, the DC amplitude (or average) of the detected
10 signal is affected, whereas the AC amplitude is not, demonstrated by a second AC wave 404. Consequently, FDPM detection involves a frequency filter, which collects only that light that is intensity modulated and therefore rejects non-modulated ambient light, as described above. The modulated fluorescent light (shown by a call-out 406) detected two centimeters away from an excited fluorophore region of
15 micromolar concentration is additionally attenuated from the source with a reduced signal of the order of approximately 10^{-4} . The changing levels of ambient light, or the average of the modulated signal (termed the DC component), will not affect modulated fluorescence amplitude. Rejection of modulated excitation light (as well as ambient light) is accomplished via appropriate selection of optical filters.

20 FIGURE 5 is the well known Jablonski diagram 500 illustrating radiative and non-radiative relaxation of fluorophores to the ground state according to an embodiment of the invention. As described above, ICG is one example of a fluorescent contrast agent that may be utilized within the teachings of the present invention. ICG has been employed as a contrast agent for surface and sub-surface
25 disease detection in small animal studies. Others have shown through immunohistochemical staining that targeted delivery of the fluorophore in the digestive tract could enable novel endoscopic detection of small cancers. Upon injecting ICG bound to purified lipoprotein into the subarachnoid space of the neonatal rat, a cooled CCD camera has been used to detect the fluorescence in the
30 subarachnoid space and cerebrospinal fluid pathways *in vivo*. The peptide transferrin has been coupled to indotricarbocyanine in order to facilitate cellular uptake and internalization into tumor cells overexpressing the transferrin receptor. More

recently, some researchers have compared the retention of ICG and its derivatized analogue, cypate, with the receptor-specific peptide-cypate conjugate (cytate) in a rat tumor model. They showed that receptor-mediated uptake results in as much as a 17:1 tumor:muscle fluorescence contrast using imaging techniques.

5 A fluorescent dye may be made lifetime-sensitive to its environment. When the dyes are conjugated tightly together within a complex, an activated fluorophore can be quenched or can relax non-radiatively by directly transferring its energy to a neighboring dye molecule, as shown in FIGURE 5. The net result is that the mean lifetime τ of the excited singlet state of the fluorophore can be comparatively
10 lengthened when compared to the fluorophore, which is free and distant from its neighboring dye molecules. Certainly, other paradigms for lifetime-sensitive fluorescent contrast agents may be envisioned, but they may require quantification of the fluorescent decay kinetics from re-emitted multiply scattered light. As the lifetime of the fluorescent dyes changes, the AC and the θ changes, enabling
15 "reporting" of an "activated" dye.

FIGURE 6 is a schematic of an example FDPM system 600 for use in imaging lymph nodes according to an embodiment of the present invention. As described above, other suitable imaging techniques may be utilized, such as the techniques described in U.S. Pat. No. 5,865,754. FIGURE 6 illustrates FDPM system 600 being
20 utilized for imaging a target body within a human. The present invention contemplates more, less, or different components than those shown in system 600 of FIGURE 6. In the illustrated embodiment, system 600 includes an intensity modulated light source 602 with a laser driver 604, operatively coupled to a laser diode 606 and reference frequency generator 608. Light source 602 is configured to
25 deliver intensity modulated excitation light into tissue of a body 610. The re-emitted light from body 610 is focused onto a gain modulated image intensifier 612 by a lens 614.

In one embodiment, image intensifier 612 includes a photocathode face that converts photons to electrons, a multi-channel plate ("MCP") that multiplies the
30 electronic signal by avalanche multiplication, and a phosphorescent screen that converts electrons into an optical image. Preferably, image intensifier 612 is a fast intensifier of the variety manufactured by Litton Electronics, Inc., which enables

modulation by applying a DC bias and an RF signal from an amplifier 616 between the photocathode and the MCP; however, other suitable image intensifiers may be utilized. In this example, the intensity modulation of the image from image intensifier 612 is phase-locked to laser diode 606 by a 10 MHz output signal from generator 608.

5 By modulating laser diode 606 and image intensifier 612 at the same frequency, a steady-state image results on the phosphor screen. The image from the phosphor screen is focused through an interference filter 618 on a charge coupled device ("CCD") camera 620 by lens 614. Camera 620, in the illustrated embodiment, has a 512 x 512 array of CCD detectors configured to provide a corresponding pixelated

10 image. Camera 620 may be operatively coupled to any suitable processor in order to image the lymph node of body 610.

In operation, following each acquired image, a phase delay between image intensifier 612 and laser diode 606 is induced by stepping the phase of image intensifier 612 to values between 0 and 360° with a frequency synthesizer 622 under

15 the control of a suitable processor. Since the gain modulation of image intensifier 612 and laser diode 606 occurs at the same frequency, homodyning results in a steady phosphorescent image on image intensifier 612, which is dependent upon phase. Preferably, control between synthesizer 622 and the processor is obtained by a conventional general purpose interface bus ("GPIB") 624; however, other suitable

20 interfaces may be utilized. Images from the phosphorescent screen image intensifier 612 may then be gathered at each phase delay by CCD camera 620. Incremental phase delayed images are then used to generate a map of phase-shift in intensity modulation ratio between the excitation and re-emitted light from body 610. By applying interference or suitable optical filters, the emission light may be selectively

25 separated from the excitation light and measured. Camera 620 output may be processed by any suitable processor. Optical filters 618 may be any suitable optical filters, such as a band pass filter, a long pass filter, a holographic notch filter, or any combination thereof, or any optical scheme to reject excitation enabling registration of the fluorescent signal.

30 In one embodiment, a gain-modulated image intensifier as a homodyned, multi-pixel radio frequency phase-sensitive camera was employed. The use of a gain-modulated image intensified charge-coupled camera (ICCD) was applied to FDPM

imaging of absorbing in scattering phantoms. Essentially, instead of detecting the light that has propagated to a point or series of points on the tissue surface, the ICCD system acts as an area detector, collecting the spatially distributed light re-emitted from the tissue surface. In one embodiment, the mammary chain was scanned using the ICCD area detector to collect the emitted fluorescence owing to ICG. Excitation was accomplished by illuminating the tissue surface with a 4 cm diameter expanded beam of a 20 mW, 780 nm laser that was modulated at 100 MHz. Test results in phantom studies are described below.

FIGURES 7A through 7D illustrate the DC (a.u.) and AC (a.u.) contrast images (background subtracted) that were simultaneously obtained when a 100 μ L target containing 100 fmol of ICG was positioned at increasing depths within one percent of a Liposyn[®] in increments of one centimeter from an illumination surface, using the system of FIGURE 6. The Liposyn[®] was used to mimic the optical properties of tissue. The images were obtained by subtracting the corresponding absence image taken under identical measurement conditions in order to eliminate excitation light leakage through the optical filters. Both DC and AC images track the position of the fluorescent target (when detected) as its depth (z) is moved within the planar (x-y) field of view. A long pass and band pass filter combination was used for optical filters 618 (FIGURE 6) in these studies at each target depth and the integration time was varied between 200 and 700 ms because the amount of light collected at the image intensifier photocathode varied. Consequently, the units of AC and DC cannot be directly compared at different target depths. The results show that as the distance of the target position becomes greater than one centimeter from the tissue surface, the DC (continuous wave) signal is lost, whereas sufficient signal is evident in AC measurements as deep as three centimeters. These results indicate reduced noise floor of AC over DC measurements and the impact upon detecting smaller amounts of fluorophore at greater tissue depths.

FIGURES 8A and 8B illustrate the image of a noise floor owing to excitation light gauge for both DC and AC images with identical settings and processing as that in FIGURE 7C, with the exception that the fluorescent target was not present in the body. The mean and standard deviation of the DC intensity counts were approximately 83 and 130 (a.u.) whereas those of the AC intensity counts were 48 and

17 (a.u.), below the AC contrast value in FIGURE 7C. These numbers are consistent with a lower noise floor associated with FDPM over continuous wave measurements.

Thus, as shown above, excitation light rejection is a significant barrier for the detection of increasing target depths. Consequently, reduction of the noise floor through reduction of excitation light leakage is important for increased penetration depth of fluorescence enhanced optical imaging. Fluorescence enhanced NIR optical imaging measurements provide localization comparable with lymphoscintigrams at tissue depths up to three centimeters for targets as small as five millimeters. With the developing chemistry of targeted fluorescent contrast agents, fluorescence enhanced optical imaging may have added benefit to sentinel lymph node mapping if NIR excitable contrast agents could not only locate sentinel lymph nodes but also report if cancer cells are present within the node.

Experimental depth sensitivity studies and corresponding results are also illustrated in U.S. Provisional Patent Application No. 60/395,279, filed July 12, 2002, which is herein incorporated by reference for all purposes.

Although embodiments of the invention and their advantages are described in detail, a person skilled in the art could make various alterations, additions, and omissions without departing from the spirit and scope of the present invention, as defined by the appended claims.